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Synthesis of the Protected Octadecapeptide corresponding to the Amino Acid Sequence [14—31] of Human β-Endorphin[1)]

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For the synthesis of human β-endorphin, the C-terminal protected octadecapeptide ester, Z(OMe)–Leu–Val–Thr–Leu–Phe–Lys(Z)–Asn–Ala–Ile–Ile–Lys(Z)–Asn–Ala–Tyr–Lys(Z)–Lys(Z)–Gly–Glu(OBzl)–OBzl, was synthesized by successive azide condensation of six peptide fragments; (position 14—15), (16—19), (20—21), (22—23), (24—26) and (27—31).

Keywords——endorphin; β-lipotropin; enkephalin; conventional synthesis of human β-endorphin (14—31); methanesulfonic acid procedure; hydrolysis of Ile-Ile with 3 N Tos-OH

Since the existence of an endogeneous ligand in the brain which acts as an agonist at opiate receptor sites has been pointed out by several investigators,[3)] the peptide nature of such morphin-like factors was first characterized by Hughes *et al.*[4)] in 1975 in the term of enkephalin; H-Tyr-Gly-Gly-Phe-Met-OH and H-Tyr-Gly-Gly-Phe-Leu-OH. It was soon realized that the former Met-enkephalin is present as residues 61 to 65 in β-lipotropin isolated from various mammalian pituitary glands; sheep,[5)] pig[6)] and man.[7)] Thus the possibility was pointed out that β-lipotropin may be the precursor of enkephalin and/or other pituitary peptides with morphin-like activity, termed as endorphin or endogeneous opioid peptides. Subsequently the untriacontapeptide (β-lipotropin 61—91) isolated by Li *et al.*[8)] from camel pituitary gland was found to possess significant opiate activity and was designated as β-endorphin. Its amino acid sequence was shown to be identical with that of the C-terminal 31 amino acids of ovine β-lipotropin. The respective peptide from human pituitary (human β-lipotropin 61—91) was next characterized.[9)] In addition, Guillemin *et al.*[10)] isolated from porcine pituitary, two other

1) Amino acids, peptides and their derivatives mentioned in this paper are of the L-configuration. Abbreviations used are those recommended by IUPAC-IUB Commission on Biochemical Nomenclature: *Biochem.*, **5**, 2485 (1966); *ibid.*, **6**, 362 (1967); *ibid.*, **11**, 1726, (1972). Z=benzyloxycarbonyl, Z(OMe)=*p*-methoxybenzyloxycarbonyl, Bzl=benzyl, DCC=dicyclohexylcarbodiimide, HOBT=1-hydroxybenzotriazole, TCP=2,4,5-trichlorophenyl, PCP=pentachlorophenyl, TFA=trifluoroacetic acid, DMF=dimethylformamide, MSA=methanesulfonic acid, DMSO=dimethylsulfoxide, NP=*p*-nitrophenyl.

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peptides, as well as porcine type β-endorphin;[11] the one called α-endorphin (β-lipotropin 61—76) and the other γ-endorphin (β-lipotropin 61—77). β-Endorphin from ovine and bovine origins was also characterized by Chrétien *et al.*[12] and Li *et al.*[13] respectively.

Solid phase synthesis of ovine β-endorphin was first reported by Li *et al.*[14] in 1976 and porcine α and γ-endorphin by Ling.[15] The same method was also applied to the synthesis of human β-endorphin by Li *et al.*[16] in 1977. In the consecutive two papers, we wish to report the synthesis of the untriacontapeptide corresponding to the entire amino acid sequence of human β-endorphin, which was performed in a conventional manner. The C-terminal portion of human β-lipotropin (position 40—91) was sequenced by Cseh *et al.*[7] in 1972 and it was shown that as far as the sequence from 61 to 91 is concerned, human β-endorphin has a tyrosine residue at position 27 and a glutamic acid residue at the C-terminal 31, instead of histidine and glutamine in ovine β-endorphin respectively.

In our synthesis, as illustrated in Fig. 1, amino acid derivatives bearing protecting groups removable by MSA[17] was employed; *i.e.*, Lys(Z) and Glu(OBzl). The sulphur atom of methionine was protected as the sulfoxide to prevent alkylation during the final deprotection by the MSA-anisole system.[18] As building blocks, nine peptide fragments were selected. In the first paper, the synthesis of the octadecapeptide resulted in the successive azide[19] condensation of six peptide fragments was described.

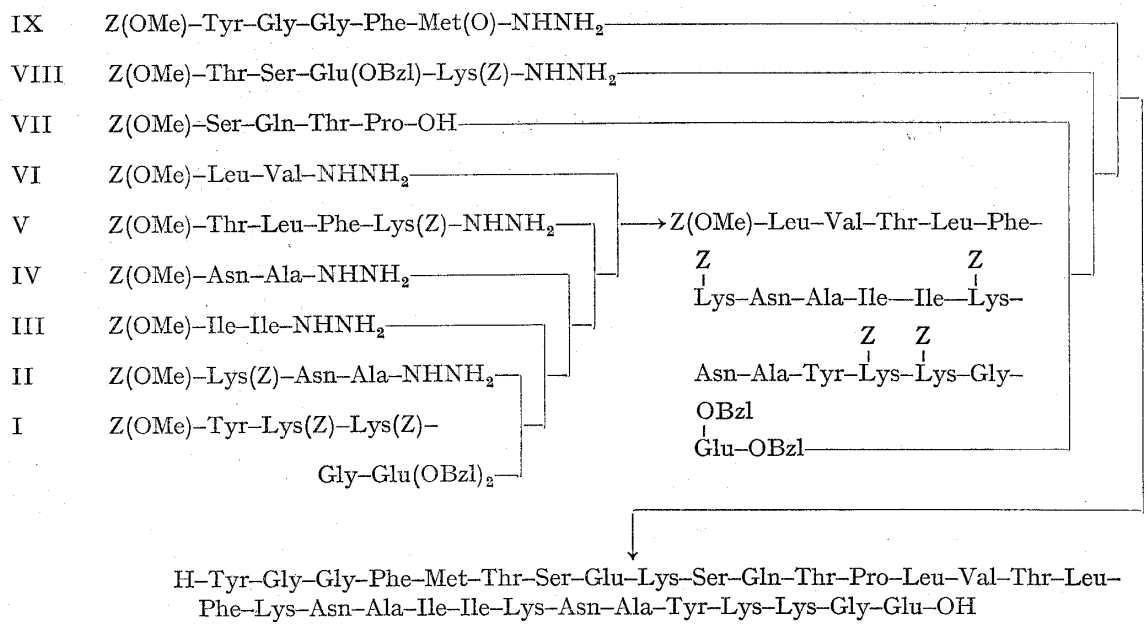

Fig. 1. Synthetic Route to Human β-Endorphin

The C-terminal protected pentapeptide, Z(OMe)-Tyr-Lys(Z)-Lys(Z)-Gly-Glu(OBzl)-OBzl (I), was synthesized according to the scheme illustrated in Fig. 2. Considering the DCC condensation[20] of the Pro-terminal peptide fragment (VII) in the latter step, the carboxyl

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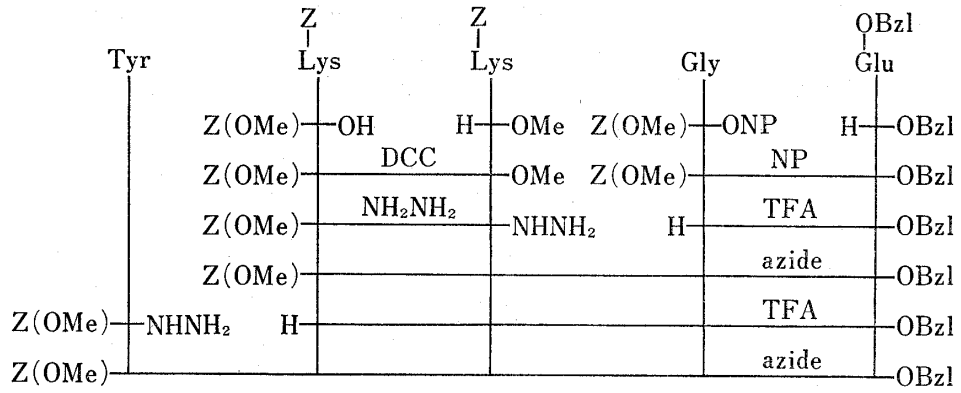

Fig. 2. Synthetic Scheme of the Protected Pentapeptide Ester (I). Z(OMe)-(β-endorphin 27—31)-OBzl

functions of the C-terminal glutamic acid were protected as the dibenzyl esters. The *p*-nitrophenyl ester procedure[21] was employed to prepare Z(OMe)-Gly-Glu(OBzl)-OBzl, from which the α-amino protecting group, Z(OMe), was removed by the usual TFA treatment.[22] The deprotected dipeptide ester was then condensed with the known dipeptide hydrazide, Z(OMe)-Lys(Z)-Lys(Z)-$NHNH_2$,[23] by the Rudinger's azide procedure[19] to give the protected tetrapeptide ester, Z(OMe)-Lys(Z)-Lys(Z)-Gly-Glu(OBzl)-OBzl. Next, the same azide procedure was applied to introduce Z(OMe)-Tyr-$NHNH_2$ and the desired pentapeptide ester (I) was purified by batchwise washing with 10% citric acid and water followed by precipitation from DMF with ethyl acetate. Such batchwise washing and precipitation procedures were employed for purification of protected peptides throughout this synthesis, when crude products were obtained as a powder by trituration with ether or ethyl acetate.

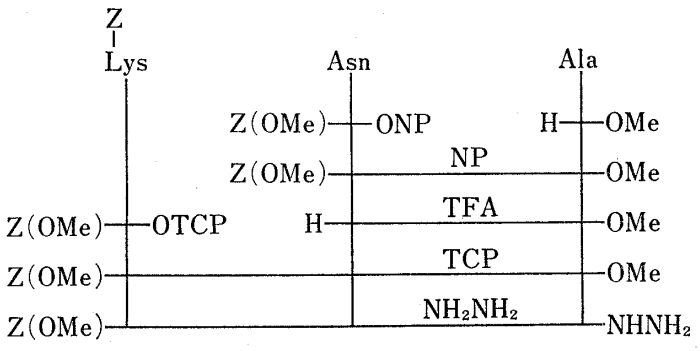

Fig. 3. Synthetic Scheme of the Protected Tripeptide Hydrazide (II). Z(OMe)-(β-endorphin 24—26)-$NHNH_2$

Next, the protected tripeptide hydrazide, Z(OMe)-Lys(Z)-Asn-Ala-$NHNH_2$ (II), was prepared starting with the known dipeptide, Z(OMe)-Asn-Ala-OMe,[24] which after α-deprotection by TFA, was allowed to condense with Z(OMe)-Lys(Z)-OH by the trichlorophenyl ester procedure[25] as shown in Fig. 3. The resulting tripeptide ester, Z(OMe)-Lys(Z)-Asn-Ala-OMe, was converted as usual to the corresponding hydrazide (II). Though this hydrazide is a small peptide derivative, it required precipitation from DMSO, instead of DMF, for purification, because of its poor solubility.

The next two fragments, Z(OMe)-Ile-Ile-$NHNH_2$ (III)[26] and Z(OMe)-Asn-Ala-$NHNH_2$ (IV)[27] are the known compounds. It seems interesting to note that the above mentioned

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tripeptide unit, Lys-Asn-Ala, occurs twice in the C-terminal portion of mammalian β-endorphin so far known. At this stage, the dipeptide hydrazide, Z(OMe)-Asn-Ala-$NHNH_2$, was selected as a fragment considering the future synthesis of δ-endorphin,[28] which is terminated at position 19. Thus the lysine residue at the 20 position was incorporated as the C-terminus into the next fragment, Z(OMe)-Thr-Leu-Phe-Lys(Z)-$NHNH_2$ (V). This protected tetrapeptide hydrazide was synthesized in a stepwise manner starting with H-Lys(Z)-OBzl as shwon in Fig. 4. The *p*-nitrophenyl ester procedure was employed for introduction of the phenylalanine residue and the pentachlorophenyl ester procedure[29] for the leucine residue respectively. In the latter step, HOBT[30] was used as an accelerant for the coupling reaction. The threonine residue was introduced by the Rudinger's azide procedure. The resulting tetrapeptide ester, Z(OMe)-Thr-Leu-Phe-Lys(Z)-OBzl, was subsequently converted to the corresponding hydrazide (V) as usual.

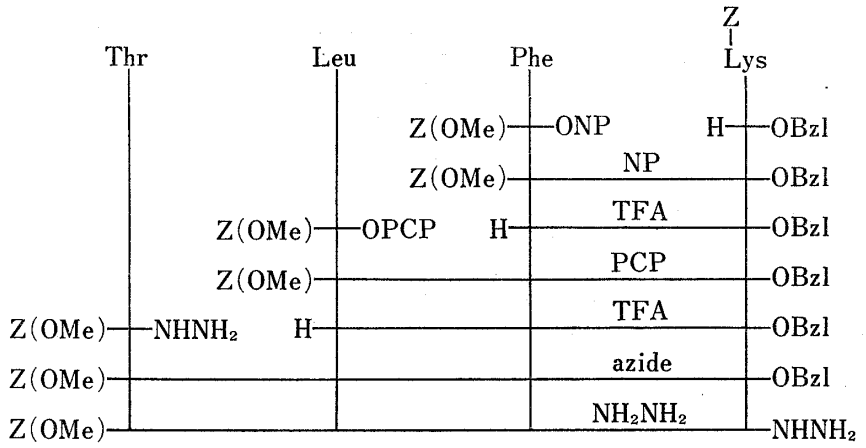

Fig. 4. Synthetic Scheme of the Protected Tetrapeptide Hydrazide (V).
Z(OMe)-(β-endorphin 16—19)-$NHNH_2$

The fragment, Z(OMe)-Leu-Val-$NHNH_2$ (VI), was synthesized as usual by the DCC condensation procedure followed by the hydrazine treatment. Somewhat prolonged exposure to hydrazine was required to convert the methyl ester, Z(OMe)-Leu-Val-OMe, quantitatively to the hydrazide (VI), because of the steric hindrance of the valine residue.

Six fragments thus obtained were then assembled according to the scheme illustrated in Fig. 1. The azide procedure was employed exclusively using the excess acyl components (1.5 equiv. each in most instances). Hydrolysis of peptides containing sterically hindered Ile-Ile residue with 3 N *p*-toluenesulfonic acid (22 hours)[31] gave poor recovery of isoleucine. Satisfactory recovery was obtained, when hydrolysis was performed with 6 N hydrochloric acid at 110° for 72 hours. As reaction solvents, DMF or DMSO–DMF was employed depending upon the solubility of the intermediates, which decreased progressively with increasing chain length. The protected products, including the protected octadecapeptide ester, Z(OMe)-Leu-Val-Thr-Leu-Phe-Lys(Z)-Asn-Ala-Ile-Ile-Lys(Z)-Asn-Ala-Tyr-Lys(Z)-Lys-(Z)-Gly-Glu(OBzl)-OBzl, were purified by batchwise washing as mentioned above followed by precipitation from appropriate solvents, such as DMF or DMSO with ethyl acetate or methanol and their purities were assessed by three criteria; thin layer chromatography, elemental analysis and acid hydrolysis. The protected octadecapeptide ester thus obtained served as an amino component for the synthesis of human β-endorphin, which will be described in the succeeding paper.

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Experimental

Melting points are uncorrected. Rotations were determined with a Union digital polarimeter PM-101. The amino acid compositions of acid hydrolysates were determined with Hitachi amino acid analyzer Model KLA-5 and values are uncorrected for amino acid destruction. Solvents were freshly distilled and evaporations were carried out *in vacuo* at a bath temperature of 40—50°. Thin-layer chromatography was performed on silicagel (Kieselgel G, Merck). *Rf* values refer to the following solvent systems: Rf_1 $CHCl_3$–MeOH–H_2O (8:3:1), Rf_2 $CHCl_3$–MeOH–AcOH (95:5:3).

Z(OMe)-Gly-Glu(OBzl)-OBzl——Z(OMe)–Gly–ONP (9.37 g) was added to a solution of H–Glu(OBzl)–OBzl (prepared from 15.07 g of the tosylate with 4.04 ml of Et_3N) in DMF (70 ml) and the mixture was stirred at room temperature for 24 hr. The solvent was evaporated and the residue was dissolved in AcOEt. The extract was washed with 5% citric acid, 5% Na_2CO_3 and H_2O–NaCl, dried over Na_2SO_4 and then evaporated. The residue was triturated with ether and recrystallized from AcOEt and ether; yield 11.21 g (79%); mp 65—67°, $[\alpha]_D^{24}$ −15.1° (c=0.4, MeOH), Rf_2 0.61. *Anal.* Calcd. for $C_{30}H_{32}N_2O_8$: C, 65.68; H, 5.88; N, 5.11. Found: C, 65.77; H, 5.88; N, 5.04.

Z(OMe)-Lys(Z)-Lys(Z)-Gly-Glu(OBzl)-OBzl——Z(OMe)–Gly–Glu(OBzl)–OBzl (21.94 g) was treated with TFA (30 ml)-anisole (9.5 ml) in an ice-bath for 60 min and the excess TFA was removed by evaporation. Treatment of the residue with *n*-hexane gave an oily precipitate, which after drying over KOH pellets *in vacuo* for 4 hr, was dissolved in DMF (15 ml) and Et_3N (11 ml) was added. To this ice-chilled solution, the azide (prepared from 14.42 g of Z(OMe)–Lys(Z)–Lys(Z)–$NHNH_2$[23] with 11.6 ml of 3.79 N HCl–DMF, 3 ml of isoamylnitrite and 9.2 ml of Et_3N) in DMF (65 ml) was added and the mixture was stirred at 4° for 48 hr. The solvent was evaporated and the residue was treated with H_2O. The resulting powder was washed batchwisely with 5% citric acid and H_2O and then precipitated from DMF with AcOEt; yield 13.90 g (65%), mp 120—121°, $[\alpha]_D^{24}$ −6.9° (c=0.5, DMF), Rf_2 0.66. *Anal.* Calcd. for $C_{58}H_{68}N_6O_{14}\cdot 1/2H_2O$: C, 64.37; H, 6.43; N, 7.77. Found: C, 64.24; H, 6.32; N, 8.12.

Z(OMe)-Tyr-Lys(Z)-Lys(Z)-Gly-Glu(OBzl)-OBzl (I)——The above tetrapeptide ester (13.50 g) was treated with TFA (15 ml)-anisole (5.4 ml) as stated above and dry ether was added. The resulting powder was dried over KOH pellets *in vacuo* for 3 hr and then dissolved in DMF–DMSO (40 ml—20 ml) containing Et_3N (2.1 ml). To this ice-chilled solution, the azide (prepared from 5.39 g of Z(OMe)–Tyr–$NHNH_2$ with 8.7 ml of 3.79 N HCl–MDF, 2.23 ml of isoamylnitrite and 8.4 ml of Et_3N) in DMF (30 ml) was added. The mixture was stirred at 4° for 48 hr, the solvent was evaporated and the residue was treated with AcOEt. The resulting powder was washed batchwisely with 10% citric acid and H_2O and then precipitated from DMF with AcOEt; yield 11.92 g (77%), mp 152—155°, $[\alpha]_D^{24}$ −12.8° (c=0.6, DMF), Rf_1 0.88. Amino acid ratios in an acid hydrolysate: Tyr 1.02, Lys 1.93, Gly 1.00, Glu 0.93 (average recovery 92%). *Anal.* Calcd. for $C_{67}H_{77}N_7O_{16}\cdot 1/2H_2O$: C, 64.61; H, 6.31; N, 7.87. Found: C, 64.59; H, 6.10; N, 8.04.

Z(OMe)-Lys(Z)-Asn-Ala-OMe——Z(OMe)–Asn–Ala–OMe[24] (6.86 g) was treated with TFA (14 ml)-anisole (6 ml) in the usual manner. The deprotected peptide precipitated by ether as a fine powder was dissolved in DMF (40 ml) containing Et_3N (5 ml) and Z(OMe)–Lys(Z)–OTCP (9.39 g) was added. The mixture was stirred at room temperature for 24 hr and the solvent was evaporated. The residue was treated with AcOEt and the resulting powder, after washing batchwisely with 5% citric acid, 5% Na_2CO_3 and H_2O, was recrystallized from $CHCl_3$ and AcOEt; yield 8.25 g (96%), mp 190—194°, $[\alpha]_D^{24}$ −13.5° (c=0.5, DMF), Rf_1 0.65. *Anal.* Calcd. for $C_{31}H_{41}N_5O_{10}$: C, 57.84; H, 6.42; N, 10.88. Found: C, 57.64; H, 6.34; N, 10.80.

Z(OMe)-Lys(Z)-Asn-Ala-$NHNH_2$ (II)——To a solution of Z(OMe)–Lys(Z)–Asn–Ala–OMe (9.16 g) in DMF (200 ml), 80% hydrazine hydrate (10 ml) was added and the mixture was kept on standing overnight. After addition of H_2O, the resulting gelatinous mass was collected by filtration, washed with MeOH and precipitated from DMSO with MeOH; yield 5.37 g (60%), mp 201—205°, $[\alpha]_D^{24}$ −13.4° (c=0.5, DMF), Rf_1 0.43. *Anal.* Calcd. for $C_{30}H_{41}N_7O_9$: C, 55.97; H, 6.42; N, 15.23. Found: C, 56.19; H, 6.46; N, 15.15.

Z(OMe)-Phe-Lys(Z)-OBzl——Z(OMe)–Phe–ONP (6.31 g) was added to a stirred solution of H–Lys(Z)–OBzl (prepared from 7.50 g of the tosylate with 2.9 ml of Et_3N) in DMF (30 ml). After 24 hr, the solution was condensed and the residue was treated with H_2O. The resulting powder was washed batchwisely with acid and base as mentioned above and recrystallized from $CHCl_3$ and ether; yield 7.20 g (75%), mp 152—155°, $[\alpha]_D^{24}$ −11.5° (c=0.4, MeOH), Rf_2 0.49. *Anal.* Calcd. for $C_{39}H_{43}N_3O_8\cdot 1/2H_2O$: C, 67.81; H, 6.42; N, 6.08. Found: C, 68.06; H, 6.35; N, 6.17.

Z(OMe)-Leu-Phe-Lys(Z)-OBzl——Z(OMe)–Phe–Lys(Z)–OBzl (6.82 g) was treated with TFA (7.6 ml)-anisole (3.2 ml) as usual and dry ether was added. The resulting powder was then dissolved in DMF (40 ml), to which Et_3N (1.4 ml) HOBT (1.35 g) and Z(OMe)–Leu–OPCP (5.44 g) were successively added. The mixture was stirred at room temperature for 24 hr, the solvent was evaporated and the residue was treated with ether. The resulting powder was washed batchwisely as stated above and then recrystallized from THF and ether; yield 4.26 g (54%), mp 146—149°, $[\alpha]_D^{24}$ −21.4° (c=0.4, DMF), Rf_2 0.78. *Anal.* Calcd. for $C_{45}H_{54}N_4O_9$: C, 67.99; H, 6.85; N, 7.05. Found: C, 67.86; H, 6.84; N, 7.15.

Z(OMe)-Thr-Leu-Phe-Lys(Z)-OBzl——Z(OMe)–Leu–Phe–Lys(Z)–OBzl (3.91 g) was treated with TFA (6 ml)-anisole (2 ml) as usual and dry ether was added. The resulting powder was dissolved in DMF (30 ml). To this solution Et_3N (0.7 ml) and the azide (prepared from 1.78 g of Z(OMe)–Thr–$NHNH_2$ with 3.5 ml

of 3.79 N HCl–DMF, 0.89 ml of isoamylnitrite and 2.76 ml of Et_3N) in DMF (25 ml) were added. The mixture was stirred at 4° for 48 hr and then condensed. The residue was treated with AcOEt and the resulting powder, after washing batchwisely as mentioned above, was precipitated from DMF with AcOEt; yield 3.31 g (74%), mp 159—161°, $[\alpha]_D^{24}$ −20.9° (c=0.4, DMF), Rf_1 0.82. *Anal.* Calcd. for $C_{49}H_{61}N_5O_{11}\cdot 1/2H_2O$: C, 65.02; H, 6.91; N, 7.74. Found: C, 64.82; H, 6.83; N, 7.51.

Z(OMe)-Thr-Leu-Phe-Lys(Z)-NHNH$_2$ (V)——To a solution of Z(OMe)–Thr–Leu–Phe–Lys(Z)–OBzl (3.29 g) in DMF (100 ml), 80% hydrazine hydrate (3.7 ml) was added. After standing overnight, the solution was diluted with H_2O and the resulting mass, after washing with MeOH, was precipitated from DMF with MeOH; yield 2.47 g (81%), mp 209—214°, $[\alpha]_D^{24}$ −14.6° (c=0.3, DMSO), Rf_1 0.61. *Anal.* Calcd. for $C_{42}H_{57}N_7O_{11}$: C, 61.52; H, 7.01; N, 11.96. Found: C, 61.22; H, 6.92; N, 12.43.

Z(OMe)-Leu-Val-OMe——DCC (20.63 g) was added to a solution of Z(OMe)–Leu–OH (29.53 g) and H–Val–OMe (prepared from 16.80 g of the hydrochloride with 14 ml of Et_3N) in DMF (50 ml)–THF (100 ml). After stirring at room temperature for 24 hr, the solution was filtered and the filtrate was condensed. Treatment of the residue with ether afforded a powder, which was washed batchwisely as mentioned above and then recrystallized from AcOEt and ether; yield 31.37 g (77%), mp 92—94°, $[\alpha]_D^{24}$ −29.1° (c=0.4, MeOH), Rf_2 0.83. *Anal.* Calcd. for $C_{21}H_{32}N_2O_6$: C, 61.74; H, 7.90; N, 6.86. Found: C, 61.76; H, 7.82; N, 6.72.

Z(OMe)-Leu-Val-NHNH$_2$ (VI)——To a solution of Z(OMe)–Leu–Val–OMe (12.77 g) in MeOH (200 ml), 80% hydrazine hydrate (16 ml) was added. After standing for 48 hr, the resulting mass was washed with MeOH and then precipitated from DMF with 50% aqueous MeOH; yield 11.08 g (87%), mp 203—207°, $[\alpha]_D^{24}$ −4.6° (c=0.5, DMF), Rf_1 0.67. *Anal.* Calcd. for $C_{20}H_{32}N_4O_5$: C, 58.80; H, 7.90; N, 13.72. Found: C, 58.73; H, 7.71; N, 13.89.

Z(OMe)-Lys(Z)-Asn-Ala-Tyr-Lys(Z)-Lys(Z)-Gly-Glu(OBzl)-OBzl——Z(OMe)–Tyr–Lys(Z)–Lys(Z)–Gly–Glu(OBzl)–OBzl (I) (9.89 g) was treated with TFA (9 ml)-anisole (3 ml) in the usual manner and dry ether was added. The resulting powder was collected by filtration, washed with ether, dried over KOH pellets *in vacuo* for 3 hr and then dissolved in DMF (40 ml) containing Et_3N (2.2 ml). To this ice-chilled solution, Z(OMe)–Lys(Z)–Asn–Ala–azide (derived from 5.25 g of the hydrazide with 4.7 ml of 3.79 N HCl–DMF, 1.2 ml of isoamylnitrite and 3.8 ml of Et_3N) in DMSO–DMF (10 ml—40 ml) and the mixture was stirred at 4° for 48 hr. The solvent was evaporated and the residue was treated with AcOEt. The resulting powder was washed batchwisely with 5% citric acid, 5% Na_2CO_3 and H_2O and then precipitated from DMF with MeOH; yield 9.45 g (70%), mp 197—201°, $[\alpha]_D^{24}$ −19.0° (c=0.3, DMSO), Rf_1 0.65. Amino acid ratios in an acid hydrolysate: Lys 3.04, Asp 1.10, Glu 0.88, Ala 1.09, Tyr 1.00, Gly 1.00 (average recovery 83%). *Anal.* Calcd. for $C_{88}H_{106}N_{12}O_{22}\cdot H_2O$: C, 62.10; H, 6.28; N, 9.88. Found: C, 62.13; H, 6.35; N, 10.02.

Z(OMe)-Ile-Ile-Lys(Z)-Asn-Ala-Tyr-Lys(Z)-Lys(Z)-Gly-Glu(OBzl)-OBzl——The above protected octapeptide ester (9.04 g) was treated with TFA (20 ml)-anisole (11 ml) in the usual manner and the deprotected peptide isolated as stated above, was then dissolved in DMF (70 ml) containing Et_3N (1.13 ml). To this ice-chilled solution, the azide (prepared from 3.42 g of Z(OMe)–Ile–Ile–NHNH$_2$[26] (III) with 4.75 ml of 3.79 N HCl–DMF, 1.21 ml of isoamylnitrite and 3.72 ml of Et_3N) in DMSO–DMF (10 ml—30 ml) was added and the mixture was stirred at 4° for 48 hr. The solvent was evaporated and the residue was treated with AcOEt. The resulting powder, after washing batchwisely as stated above, was precipitated from DMF with AcOEt; yield 9.03 g (88%), mp 253—256°, $[\alpha]_D^{24}$ −15.9° (c=0.3, DMSO), Rf_1 0.69. Amino acid ratios in 3 N Tos–OH hydrolysate: Ile 0.85, Lys 3.04, Asp 1.01, Ala 1.08, Tyr 1.00, Gly 1.00, Glu 0.91 (average recovery 82%). In 6 N HCl hydrolysate for 72 hr: Ile 1.72, Lys 3.14, Asp 1.04, Ala 1.05, Tyr 0.22, Gly 1.00, Glu 0.94 (average recovery 89%). *Anal.* Calcd. for $C_{100}H_{128}N_{14}O_{24}\cdot 1.5H_2O$: C, 62.00; H, 6.82; N, 10.12. Found: C, 61.81; H, 6.66; N, 9.99.

Z(OMe)-Asn-Ala-Ile-Ile-Lys(Z)-Asn-Ala-Tyr-Lys(Z)-Lys(Z)-Gly-Glu(OBzl)-OBzl——The above protected decapeptide ester (8.27 g) was treated with TFA (16 ml)-anisole (9 ml) in the usual manner and the deprotected peptide isolated as stated above, was dissolved in DMF (50 ml) containing Et_3N (1.2 ml). To this ice-chilled solution, the azide (prepared from 2.48 g of Z(OMe)–Asn–Ala–NHNH$_2$[27] (IV) with 3.78 ml of 3.79 N HCl–DMF, 0.97 ml of isoamylnitrite and 3.1 ml of Et_3N) in DMF (30 ml) was added. The mixture was stirred at 4° for 48 hr, the solvent was evaporated, the residue was treated with H_2O and the resulting powder, after washing batchwisely as stated above, was precipitated from DMF with MeOH; yield 8.05 g (89%), mp 277—281°, $[\alpha]_D^{24}$ −26.6° (c=0.4, DMSO), Rf_1 0.66. Amino acid ratios in 3 N Tos–OH hydrolysate: Asp 2.36, Ala 2.29, Ile 1.06, Lys 3.38, Tyr 0.62, Gly 1.00, Glu 0.97 (average recovery 88%). *Anal.* Calcd. for $C_{107}H_{139}N_{17}O_{27}\cdot H_2O$: C, 60.81; H, 6.73; N, 11.27. Found: C, 60.87; H, 6.60; N, 11.28.

Z(OMe)-Thr-Leu-Phe-Lys(Z)-Asn-Ala-Ile-Ile-Lys(Z)-Asn-Ala-Tyr-Lys(Z)-Lys(Z)-Gly-Glu(OBzl)-OBzl——The above protected dodecapeptide ester (5.45 g) was treated with TFA (10 ml)-anisole (5.6 ml) in the usual manner and the deprotected peptide isolated as stated above was dissolved in DMF (30 ml) containing Et_3N (0.7 ml). To this ice-chilled solution, the azide (prepared from 2.44 g of Z(OMe)–Thr–Leu–Phe–Lys(Z)–NHNH$_2$ with 1.74 ml of 3.79 N HCl–DMF, 0.45 ml of isoamylnitrite and 1.38 ml of Et_3N) in DMF (18 ml) was added. The mixture was stirred at 4° for 48 hr, the solvent was evaporated and the residue was treated with AcOEt and H_2O. The resulting powder was washed batchwisely as stated above and then precipitated from DMF with MeOH; yield 6.25 g (88%), mp 279—285°, $[\alpha]_D^{24}$ −15.1° (c=0.3, DMSO), Rf_1 0.66. Amino acid ratios in 3 N Tos–OH hydrolysate: Lys 3.97, Asp 2.41, Thr 1.00, Glu 0.93, Gly 1.02, Ala

2.14, Ile 0.75, Leu 0.98, Tyr 0.99, Phe 1.03 (average recovery 96%). *Anal.* Calcd. for $C_{140}H_{184}N_{22}O_{34}\cdot H_2O$: C, 61.43; H, 6.85; N, 11.26. Found: C, 61.35; H, 6.79; N, 11.31.

Z(OMe)-Leu-Val-Thr-Leu-Phe-Lys(Z)-Asn-Ala-Ile-Ile-Lys(Z)-Asn-Ala-Tyr-Lys(Z)-Lys(Z)-Gly-Glu-(OBzl)-OBzl, Z(OMe)-(human β-endorphin 14-31)-OBzl——The above protected hexadecapeptide ester (5.00 g) was treated with TFA (10 ml)–anisole (6 ml) as usual and the deprotected peptide isolated as stated above was dissolved in DMF–DMSO (30 ml—10 ml) containing Et_3N (0.3 ml). To this ice-chilled solution, the azide (prepared from 1.13 g of Z(OMe)–Leu–Val–$NHNH_2$ with 1.60 ml of 3.79 N HCl–DMF, 0.41 ml of isoamylnitrite and 1.27 ml of Et_3N) in DMF (10 ml) was added. After stirring at 4° for 48 hr, the solvent was evaporated, the residue was treated with AcOEt and H_2O and the resulting powder, after washing batchwisely as stated above, was precipitated from DMSO with MeOH; yield 4.89 g (91%), mp 306—312°, $[\alpha]_D^{24}$ −19.2° (c=0.3, DMSO), Rf_1 0.67. Amino acid ratios in 3 N Tos–OH hydrolysate; Lys 4.14, Asp 2.30, Thr 1.01, Glu 0.93, Gly 1.00, Ala 2.03, Val 0.87, Ile 0.79, Leu 1.88, Tyr 0.97, Phe 1.06 (average recovery 94%). *Anal.* Calcd. for $C_{151}H_{204}N_{24}O_{36}\cdot 4H_2O$: C, 60.38; H, 7.11; N, 11.19. Found: C, 60.17; H, 6.71; N, 11.32.

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